

University of Groningen

**Cardioprotection with the Ca-Entry Blocker, Diltiazem. Monitoring of Energy Metabolism with  $^{31}\text{P}$ -NMR and Assay of Myocardial Loss of Nucleosides.**

Gilst, W.H. van; Robillard, G.T.; Wildevuur, Ch.R.N.

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*

Publisher's PDF, also known as Version of record

*Publication date:*

1985

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

Gilst, W. H. V., Robillard, G. T., & Wildevuur, C. R. N. (1985). *Cardioprotection with the Ca-Entry Blocker, Diltiazem. Monitoring of Energy Metabolism with  $^{31}\text{P}$ -NMR and Assay of Myocardial Loss of Nucleosides..*

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

*Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.*

**ASCORBIC ACID: ITS CELLULAR TRANSPORT CHARACTERISTICS**

Maikel Raghoobar, Jan A.M. Huisman and Cees A.M. van Ginneken

The cellular pharmacokinetics of L-ascorbic acid (AA) is especially relevant in view of the direct effects of this vitamin on cell, notably leucocyte, function. The silicone-oil cushion method that we used previously to determine the cell association of salicylic acid (1) also appears to be suitable for quantitative determination of the cellular association of ( $^{14}$ C)-AA and its oxidized form ( $^{14}$ C)dehydro-ascorbic acid (DHA). As these compounds are labile in aqueous solutions, it was necessary to perform all experiments according to very strict protocols. Further concurrently with all radiochemical experiments spectrophotometric observations were done in order to establish the enzymatic and nonenzymatic oxidation rate of AA and the intactness of the cells. The degree of cell association of AA (extracellular concentration range of 10 to 140  $\mu$ M) turned out to be 1-3 % to polymorphonuclear leucocytes (PMNs), 0.4-1.2 % to mononuclear leucocytes and 0-0.2 % to erythrocytes. PMN accumulation of DHA (3-4 %) exceeded that of AA considerably. The percentual association of AA to PMNs was dependent on extracellular concentration of AA (with an apparent  $K_d$  of about 44  $\mu$ M), incubation temperature (association being lower at 4 than at 37  $^{\circ}$ C), intactness of the cells (lysis caused 50 to 80 % decrease) and the extracellular pH (optimum at pH 6). We suggest that the association process comprises at least one saturable pathway. This is corroborated by the significant inhibition of total cellular association by probenecid. Interestingly, we found that activation of PMNs by the tumor promoter phorbol myristate acetate exaggerates the accumulation of AA threefold.

The demonstration of a substantial association of AA to PMNs is especially relevant in view of the fact that it is generally accepted that mainly DHA is taken up by PMNs.

I. M. Raghoobar et al, *Biochem. Pharmacol.* 33 (1984) 2937.

Department of Pharmacology, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

**CARDIOPROTECTION WITH THE Ca-ENTRY BLOCKER, DILTIAZEM. MONITORING OF ENERGY METABOLISM WITH  $^{31}$ P-NMR AND ASSAY OF MYOCARDIAL LOSS OF NUCLEOSIDES.**

F.D. Rahusen, W.H. van Gilst, G.T. Robillard, Ch.R.N. Wildevuur.

Earlier studies have shown improved 24 hrs hypothermic cardiac preservation with diltiazem by protection during reperfusion. This was shown by reduced overflow of adenosine during reperfusion. Total purine overflow in the first minute of reperfusion was comparable to control hearts. Therefore, it is uncertain whether diltiazem exerts any effect during ischemia. In this study nuclear magnetic resonance was used to quantitate high energy phosphates and intracellular pH in order to investigate the processes during ischemia. Isolated rat hearts were maintained in hypothermic ischemia for 6 hrs after a perfusion according to Langendorff. NMR measurements were done at 1 hr intervals. Subsequently hearts were reperfused and the coronary effluent was collected for assay of nucleosides. Diltiazem (30  $\mu$ M) treatment was started 15 minutes before ischemia (n=6). Six hearts served as controls. The results showed no significant differences in NMR measurements; in both groups pH was lowered to  $6.01 \pm 0.10$  for control and  $6.08 \pm 0.07$  for treated hearts and ATP had disappeared after 4 hrs. In relation to these findings there were also no significant differences in total purine overflow during the first minute of reperfusion ( $1529 \pm 144$  nM/min.gdw for control group and  $1570 \pm 193$  nM/min.gdw for treated group). Control hearts showed delayed adenosine overflow due to reperfusion induced damage (max. overflow  $25 \pm 10$  nM/min.gdw). This was abolished by diltiazem treatment. It is concluded that: 1) NMR is a very useful tool to monitor high energy phosphates and correlates well with the total purine overflow in the coronary effluent. 2) diltiazem is indeed effective only during reperfusion.

Depts of Clinical Pharmacology/Experimental Surgery/Physical Chemistry, Bloemsingel 1, 9713 BZ Groningen, The Netherlands.

**ISOLATION AND ORGANIC ANION TRANSPORT PROPERTIES OF MEMBRANE VESICLES FROM DOG RENAL CORTEX**

F.G.M. Russel, P.E.M. van der Linden, W.G. Vermeulen and C.A.M. van Ginneken

Isolated membrane vesicles have become a powerful tool in the study of renal transport mechanisms. In order to support our *in vivo* investigations on the renal handling of arylcarboxylic acids and their conjugates we developed an isolation procedure for brush border (BBMV) and basolateral membrane vesicles (BLMV) from dog kidney.

BBMV and BLMV were isolated simultaneously from proximal tubular cells of the renal cortex. BBMV were prepared by a  $\text{CaCl}_2$ -precipitation method and membrane suspensions enriched for BLMV were isolated with a self-orienting Percoll gradient. The enzyme markers for BBMV, alkaline phosphatase, and for BLMV, Na-K-ATPase, were enriched from the crude homogenate 10- to 12-fold and 8- to 10-fold respectively. Only little cross contamination was observed.

The uptake of D- $^3\text{H}$ /glucose and p-amino/ $^3\text{H}$ /hippurate (PAH) in these vesicles was studied by a rapid filtration technique. BLMV were free of D-glucose transport and showed a saturable sodium coupled PAH uptake sensitive to probenecid. A rapid sodium dependent D-glucose uptake was observed in BBMV. PAH uptake in these vesicles could be stimulated both by a sodium gradient and an inwardly directed  $\text{H}^+$ -gradient. Interestingly, pH stimulated transport was more sensitive to probenecid and showed higher initial uptake rates. Isolated membranes could be stored for several weeks at  $-80^{\circ}\text{C}$  without loss of D-glucose and PAH transport activity. However, despite the findings of several authors, vesicles isolated from frozen cortex showed poor transport properties.

The substrate specificity of the PAH transporter in BBMV and BLMV is currently studied by competition experiments with a series of substituted arylcarboxylic acids and their glycine conjugates.

Department of Pharmacology, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

**ARE CATECHOLAMINERGIC AND SEROTONERGIC NEURONS INVOLVED IN UPTAKE AND RELEASE OF HISTAMINE BY RAT BRAIN SLICES?**

R.P.J.M. Smits, H.W.M. Steinbusch and A.H. Mulder.

Substantial evidence supports the view that histamine (HA) functions as a neurotransmitter in the CNS. Analogous to other monoaminergic neuronal systems a specific high affinity (re-)uptake mechanism for HA into histaminergic neurons might be expected to exist, but direct evidence for this is lacking. Nevertheless, we have shown previously, that  $^3\text{H}$ -HA is taken up by brain slices and can subsequently be released by depolarizing stimuli. However, although it was demonstrated that  $^3\text{H}$ -HA release originates from neurons, it still remains uncertain, whether or not exclusively histaminergic neurons are involved. Therefore, uptake and/or release of  $^3\text{H}$ -HA as well as that of  $^3\text{H}$ -noradrenaline (NA),  $^3\text{H}$ -5HT and  $^3\text{H}$ -dopamine (DA) by slices from different rat brain regions were studied after various pharmacological treatments. Pretreatment with 6-hydroxydopamine (6-OH-DA), destroying catecholaminergic nerve terminals, lowered  $^3\text{H}$ -NA uptake (hippocampus) by 90% whereas  $^3\text{H}$ -HA uptake remained unchanged. On the other hand, 6-OH-DA lesions resulted in a decreased uptake of both  $^3\text{H}$ -DA (-85%) and  $^3\text{H}$ -HA (-40%) by striatal slices. Similarly, pretreatment with 5,7-dihydroxytryptamine (5,7-DHT), destroying serotonergic nerve terminals, reduced the uptake of both  $^3\text{H}$ -5HT (-70%) and  $^3\text{H}$ -HA (-40%) by hippocampal slices. Immunocytochemical data indicated that histaminergic neurons were not affected by either 6-OH-DA or 5,7-DHT. The data suggest that, whereas uptake of  $^3\text{H}$ -HA into noradrenergic neurons seems unlikely, dopaminergic and serotonergic neurons may contribute to a significant extent to the uptake of  $^3\text{H}$ -HA in brain slices. This conclusion is further supported by the results of experiments examining whether the electrically evoked release of  $^3\text{H}$ -HA, like that of  $^3\text{H}$ -NA (cortex),  $^3\text{H}$ -DA (striatum) and  $^3\text{H}$ -5HT (hippocampus), is subject to presynaptic modulation by activation of NA-, DA-, 5HT- or opioid receptors.

Dept. of Pharmacology, Free University Medical Faculty, Van der Boechorststraat 7, 1081 BT Amsterdam.